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PROGRAM
and
ABSTRACTS

21st PLANT DEVELOPMENT WORKSHOP

UNIVERSITY of WATERLOO

1988

TWENTY-FIRST PLANT DEVELOPMENT WORKSHOP

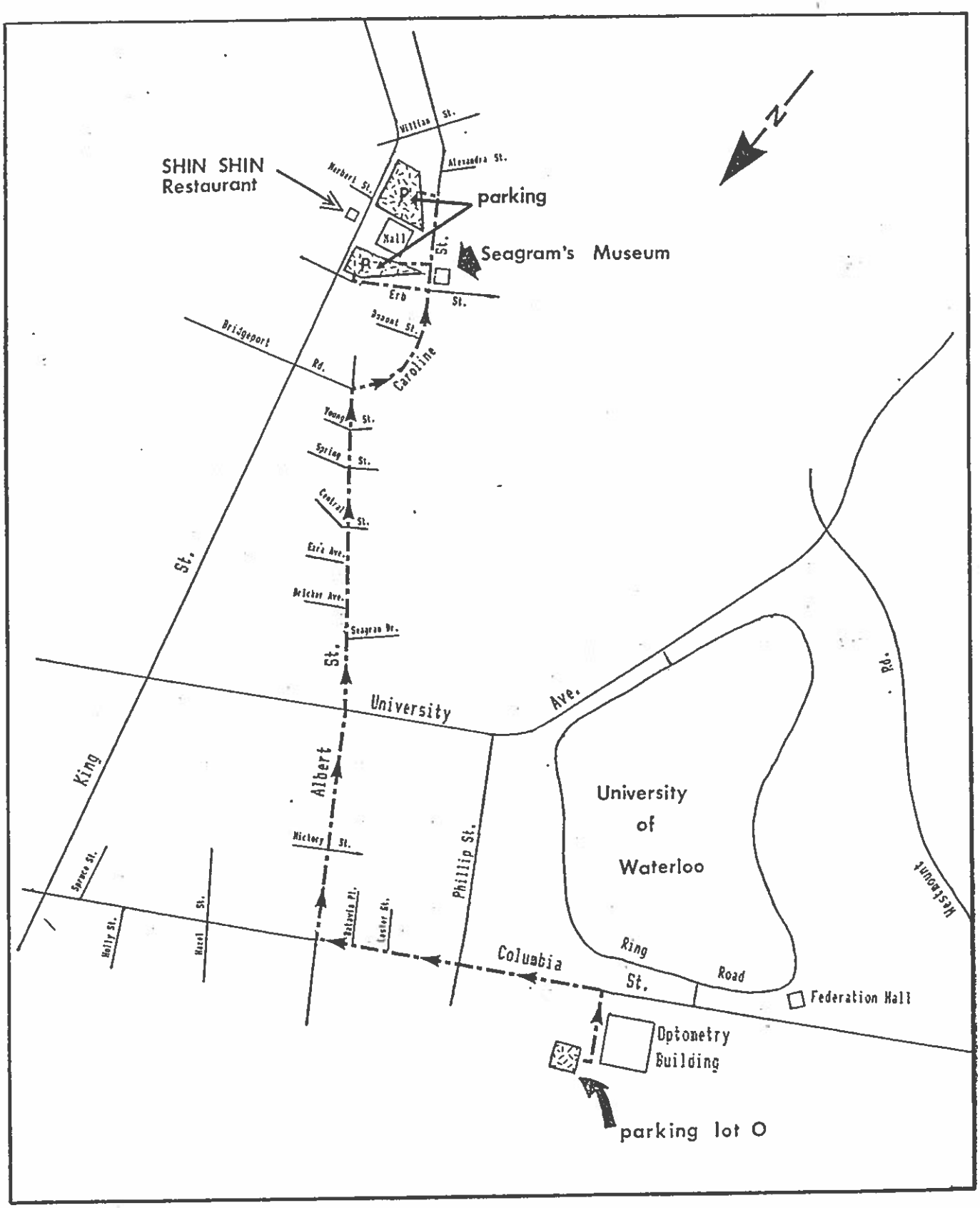
Saturday, May 14, 1988
University of Waterloo

PROGRAM

	9:00- 9:30	Registration
1	9:30- 9:45	GARRIOCK, M.L., R.L. PETERSON and C.A. ACKERLEY Early stages in colonization of Allium porrum L. cv. Titan roots by the VAM fungus, Glomus versiforme.
2	9:45-10:00	BRUNDRETT, M. and B. KENDRICK. The influence of root anatomy on mycorrhizal colonization.
3	10:00-10:15	YIP, M. and U. POSLUSZNY. Floral morphology of the genus Zostera.
4	10:15-10:30	DENGLER, N.G. Effect of shading on the expression of anisophylly in Paradrymonia ciliosa (Gesneriaceae).
	10:30-11:00	break
5	11:00-11:15	KHAYAT, E., E.B. DUMBROFF and B.R. GLICK. Effects of high light intensities on protein and chlorophyll turnover in leaves of sorgum and corn.
6	11:15-11:30	REGAN, S.M. and B.R. GLICK. Transcriptional regulation of carbon and nitrogen fixation in the Cyanobacterium Anabaena variabilis.
7	11:30-11:45	CHAPPLE, C., J. GLOVER and B.E. ELLIS. Purification of a methionine aminotransferase from Brassica carinata seedlings.
8	11:45-12:00	ENSTONE, D.E. and C.A. PETERSON. Restrictions to apoplastic influx in root apices.
	12:00- 2:00	lunch and POSTERS
9	2:00- 2:15	DIXON, M.A. and C.A. PETERSON. Why don't cut roses last longer?
10	2:15- 2:30	HUDAK, K. and D.R. LEE. Morphometric analysis of the vascular changes in the pedicel of the tomato from fruit set to maturity.
	2:30- 3:00	break
11	3:00- 4:00	ALONI, R. The control of organized differentiation of vascular tissues in plants.

4:30 Tour of Seagram Museum

6:00 Dinner at Shin Shin Chinese Restaurant



ABSTRACTS

PAPERS

1 Early stages in colonization of Allium porrum L. cv. Titan roots by the VAM fungus, Glomus versiforme

GARRIOCK, M.L., R.L. PETERSON and C.A. ACKERLEY
Department of Botany, University of Guelph, Guelph, Ontario N1G 2W1.

Leek seedlings were inoculated by the VAM fungus Glomus versiforme and stages in colonization of roots examined. External hyphae had a single-layered wall surrounding a vacuolate cytoplasm. Intra-hyphal hyphae were frequent in the extraradical mycelium. Some external hyphae formed simple, lenticular, appressoria over the outher tangential wall of epidermal cells. Appressoria were multinucleate and possessed many structures which we have interpreted to be bacterium-like organelles (BLO's). Infection pegs formed by appressoria initially grew between adjacent epidermal cells and then penetrated the radial cell walls of contiguous epidermal cells. Infection pegs were surrounded by an electron dense substance and host cell wall. In some cases the appressorium and one of the infection pegs were separated by a septum from the other infection peg. The appressorium and the infection peg separated by a septum subsequently degenerated. Hyphae in the first cortical cell layer formed coils. These hyphae, which were surrounded by host plasma membrane but not host cell wall, were multinucleate and contained many BLO's.

2 **The Influence of Root Anatomy on Mycorrhizal Colonization**

Mark Brundrett and Bryce Kendrick
Department of Biology,
University of Waterloo

Root anatomy features may have the potential to influence or even regulate colonization by mycorrhizal fungi. For example, suberin, which is found in the exodermis of many species, is known to act as a barrier to fungi, potentially limiting their ingress to younger regions of the root. To examine possible interactions between root anatomy on the one hand, and initial penetration and subsequent spread of VAM fungi in roots on the other, root morphology of five common herbaceous woodland plants was examined in detail. The time-course of mycorrhizal events and the development of root anatomy features was also compared. It was found that mycorrhiza formation often paralleled the development of root anatomy features, especially the exodermis. Several species had an exodermis with uniform exodermis suberization which developed just after fungal penetration. In plants such as Trillium and Smilacina, which have a dimorphic exodermis, long cells developed prior to mycorrhizal fungus penetration, which always occurred through an unsuberized passage cell. It is not known if suberin in exodermal cells is an absolute barrier or only a hindrance to root penetration by VAM fungi.

3 Floral morphology of the genus *Zostera*

M. YIP* and U. POSLUSZNY
Department of Botany, University of Guelph, Guelph, Ont. N1G 2W1

The floral morphology of four species of *Zostera* were examined using light, epi-illumination light and scanning electron microscopy. *Zostera* is monoecious. Flowers are borne on a flattened 'spadix' which, in turn, is enclosed by the 'spathe'. Female parts are reduced to carpels, each with two large stigmas while male parts are reduced to two sessile anthers. Male and female parts alternate in two rows on the 'spadix'. Retinacules are present in the subgenus, Zosterella. These have been variously interpreted as bracts, reduced perianth parts or appendages of the connectives of stamens. Comparisons will be made with the monotypic genus, *Heterozostera* (Zosteraceae), which is distinguished from *Zostera* by two main criteria; sympodial rhizome growth and numerous cortical bundles.

4 EFFECT OF SHADING ON THE EXPRESSION OF ANISOPHYLLY IN PARADRYMONIA CILIOSA (GESNERIACEAE)

Nancy G. Dengler, Department of Botany, University of Toronto

The expression of anisophylly in Paradrymonia ciliosa is variable: nodes of an individual shoot may have either anisophyllous pairs of laminar and linear leaves or isophyllous pairs of either leaf type. This study examines the effect of shading on leaf expansion and on the expression of anisophylly as indicated by the ratio and difference between lengths of the leaves of a pair. Under both light treatments, leaf primordia of a pair are similar at P1. Expansion of laminar leaves continues for six to seven plastochrons, while growth of the linear leaves is arrested at the P2 - P3 stage. Under reduced irradiation, a greater proportion of leaf primordia develops as linear leaves, and those that develop as laminar leaves are slightly larger. This bimodal distribution of leaf form indicates that light level has a threshold effect on the regulation of leaf expansion in this species.

5 EFFECTS OF HIGH LIGHT INTENSITIES ON PROTEIN AND CHLOROPHYLL TURNOVER IN LEAVES OF SORGHUM AND CORN.

E. Khayat, E.B. Dumbroff and B.R. Glick. Dept. of Biol. Univ. of Waterloo, Ont., N2L 3G1, Canada.

Two-week-old seedlings of sorghum and corn previously grown in the dark for 12h were exposed to low, medium or high levels of white light in a growth chamber for 24h. Changes in total protein and CHL (chlorophyll) followed similar trends characteristic of each species at all light intensities throughout the test period. High light induced sharp reductions in the concentrations of both constituents within the first 8-12h of the light phase. Exposure to medium light intensity caused similar effects in sorghum, but in corn, protein and CHL levels rose substantially during the initial 4-8h of light and then returned to levels characteristic of late dark phase. In contrast, analysis of ^{35}S-methionine-labelled leaf tissue showed that high light stimulated protein synthesis during the early portion of the light phase in sorghum but severely inhibited synthesis in corn. Fluorograms of SDS gels supported these observations. High light caused a marked and rapid decline in synthesis of RuBP and PEP carboxylase in corn, but in sorghum the synthesis of both enzymes increased substantially before a sharp decline that began 4-6h after exposure to the high light regime.

6 Transcriptional Regulation of Carbon and Nitrogen Fixation in the Cyanobacterium Anabaena variabilis. Sharon M. Regan and Bernard R. Glick, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

The diazotrophic and filamentous cyanobacterium A. variabilis was grown axenically in an airlift fermentor under conditions of constant temperature and pH. The levels of light, carbon dioxide and added fixed nitrogen used in these growth experiments was varied systematically. The effect of these changes in growth conditions on i) the growth rate, ii) the frequency of heterocyst formation and iii) the steady-state transcription of ribulose bisphosphate carboxylase, phosphoenolpyruvate carboxylase and nitrogenase genes was examined. The growth rate was monitored by periodically measuring the absorbance of the growth medium at 600 nm. The frequency of heterocyst formation was determined by microscopic examination of late log cells. Transcription was measured by RNA slot blot hybridizations between A. variabilis total RNA and radiolabelled heterologous gene specific DNA probes. The results will be discussed in terms of the regulation of carbon and nitrogen fixation in A. variabilis emphasizing the interaction between these mutually dependent but physically separated processes.

7 PURIFICATION OF A METHIONINE AMINOTRANSFERASE FROM BRASSICA CARINATA SEEDLINGS.

C. CHAPPLE, J. Glover, and B.E. Ellis
Department of Chemistry and Biochemistry,
University of Guelph,
Guelph, Ontario N1G 3P5

The transamination of methionine is considered to be the first step in alkylglucosinolate biosynthesis in Brassica. This same enzymatic reaction also functions in the reverse direction as part of a sulphur salvage pathway, now known as the methionine cycle, following ethylene and polyamine synthesis. The isolation of a methionine aminotransferase from B. carinata was undertaken to obtain probes for examining both of these metabolic functions, with the ultimate goal being the modulation of glucosinolate biosynthesis in Brassica. A 220 kD methionine:glyoxylate aminotransferase has been partially purified from leaf tissue of B. carinata, using chromatofocussing, gel filtration, and ion exchange chromatography on Mono Q. Ion exchange chromatography resolves the enzyme into 3 forms. The potential role of each of these putative isoenzymic forms in glucosinolate biosynthesis or the methionine cycle is currently under investigation.

8 Restrictions to apoplastic influx in root apices

Daryl E. Enstone and Carol A. Peterson
Department of Biology, University of Waterloo.

Previous applications of apoplastic tracers to roots delimited the accessible regions in mature zones. However, the situation in root tips remained confused; some studies indicated that the apex was completely permeable while others demonstrated regions of various sizes which excluded tracers. A tracer system employing the fluorescent alkaloid berberine and a precipitant, potassium thiocyanate, was developed and applied to mature and apical root zones. Three non-exodermal (pea, broad bean, and sunflower) and two exodermal (corn and onion) species were tested. The presence of endodermal and exodermal Casparian bands in mature primary roots was confirmed. Apical regions are capable of restricting apoplastic influx but there is no discrete, localized barrier such as a Casparian band. The exclusion zone encompassed the meristem and adjacent tissue, including the central root cap of corn and possibly sunflower. Apical apoplastic resistance may result from a low porosity of young cell walls and from the secretory nature of the root tip.

9 WHY DON'T CUT ROSES LAST LONGER?

1M.A. Dixon* and 2C.A. Peterson. 1Dept. of Hort. Science, Univ. of Guelph, Guelph, Ontario, N1G 2W1. 2Dept. of Biology, Univ. of Waterloo, Waterloo, Ontario.

Microscopic examination of the xylem of cut rose stems showed bacterial blockage appearing within 100 mm from the cut end during the first 3-5 days. This was shown to initiate a drop in stem water potential. Thereafter, increase in the resistance to water flow in the stem was found to be induced by cavitation of the water column in the xylem rather than physical blockage. Loss of functional xylem due to cavitation was shown by drawing a fluorescent tracer through the stem. Cavitated vessels did not stain with the dye and showed no physical blockage. As the level of water stress increased with time, the rate of cavitation also increased leading, in turn, to increased resistance to water flow.

10 MORPHOMETRIC ANALYSIS OF THE VASCULAR CHANGES IN THE PEDICEL OF THE TOMATO FROM FRUIT SET TO MATURITY

Kathi Hudak and D.R.Lee, Biology Department, Memorial University of Newfoundland, St. John's, Nfld., A1B 3X9

Serial sections of the pedicels of tomato fruit were taken at weekly intervals from the time of fruit set to maturity. Sections were stained, mounted and photographed then analyzed for the changes in cross sectional area of vascular tissues which occur in the abscission zone located in the "knuckle" in the pedicel.Total phloem cross sectional area increased from approximately 25% to at least 50% of total cross sectional area, while there was a coincident reduction in the xylem cross sectional area from approximately 30% to 1- 10%. The bicollateral nature of the phloem is lost in the abscission zone as the xylem cylinder is reduced to a few vessels dispersed through the increased cross sectional area of phloem. This pattern of vascular change remained consistent throughout the growth period. The site of this vascular transition will be where the water demand of the fruit will be transferred to the phloem, and will link the movement of water and dry matter to the fruit.

11 THE CONTROL OF ORGANIZED DIFFERENTIATION OF VASCULAR TISSUES IN PLANTS

Roni Aloni
Department of Botany, Tel Aviv University, Tel Aviv 69978, Isreal

The aim of this lecture is to present current thoughts and evidence on the role of both endogenous and exogenous factors in controlling the organized differentiation of vascular tissues. It will focus on the role of auxin as it seems to be the major signal involved in all aspects of vascular differentiation (Roberts et al. 1988). The following problems will be discussed: control of vascular strand formation, differentiation of circular vessels, relationship between phloem and xylem, control of conduit size and density, and the control of vascular adaptation (Aloni 1987).

Aloni, R. 1987. Differentiation of vascular tissues. Ann. Rev. Plant Physiol. 38: 179-204.

Roberts, L.W., Gahan, P.B., Aloni R. 1988. In: Springer Series in Wood Science. Vol. 6. Vascular Differentiation and Plant Growth Regulators. ed T.E. Timell. Berlin, Heidelberg, New York: Springer Verlag.

POSTERS

ETHYLENE PRODUCTION AND EMBRYOGENESIS IN BARLEY (Hordeum vulgare) ANTHER CULTURE.

U. H. CHO and K. J. KASHA, Department of Crop Science, University of Guelph, Guelph, Ontario, N1G 2W1

The relationship between ethylene gas production from anthers cultured in vitro and embryogenesis in barley (Hordeum vulgare) was studied. Ethylene gas production was detected just after inoculation, being the highest within 24 h and not detected after 36 h. The plantlet forming callus produced more ethylene than non-plantlet forming callus. Ethylene gas was assumed to be from endogenous 1-aminocyclopropane-1-carboxylic acid (ACC) and triggered by senescence of anthers and auxin (2,4-D) added in the media. The ethylene was thought to be necessary for induction of abnormal cell divisions leading to callus production in anther culture. Further studies on the relationship between ethylene gas and callus production from different genotypes are in progress.

NITROGEN, PHOSPHATE AND STARCH STORAGE IN ERIOPHORUM VAGINATUM, AN ARCTIC SEDGE

Marilyn Griffith, Biol. Dept., Univ. of Waterloo, Waterloo, ONT, Canada N2L 3G1 & Linda Defoliart, Inst. of Arctic Biol., Univ. of Alaska, Fairbanks, AK 99775-0180 USA

In E. vaginatum, the stem below the current leaves is modified for nutrient storage & was studied anatomi-cally using light and electron microscopy. An unusual vascular structure permits rapid nutrient exchange between current leaves, current roots, and the stem. Nitrogen and phosphorus are limiting nutrients in the tundra and are extensively recycled within the plant. Biochemical and histochemical techniques were used to identify forms and location of nutrient storage. N, mainly in the form of ARG, ASN and GLN, is stored overwinter in protein bodies located in parenchyma cells associated with the vascular tissue of the stem. P appears to be stored as polyphosphate in the endodermoid layer and in transfer cells associated with stem xylem. Carbohydrate is not limiting & is stored as starch in less accessible cortical cells.

IS THE TEMPERATURE SENSITIVITY OF TRANS-16:1/16:0 IN RYE THYLAKOID PHOSPHATIDYLGLYCEROL DEPENDENT ON LHCII INSERTION? M. Krol, N.Huner, Dept. Plant Sciences, Univ. of Western Ontario, London, ONT, and J.P. Williams, E. Maisson, Botany Dept., Univ. Toronto, ONT.
Our previous results indicated that development of rye (Secale cereale L.) results in a specific 70% decrease in the trans-16:1 content of thylakoid PG which modulates the organization of LHCII such that the monomer predominates at 5°C whereas the oligomer predominates at 20°C. Furthermore, PG specifically was shown to be physically associated with LHCII. To determine whether the light or temperature sensitive accumulation of trans-16:1 requires the insertion of LHCII into the thylakoid membrane, etiolated rye seedlings developed at 5°C (RH) or 20°C (RNH) were subjected to chloroplast development under intermittent light conditions (IML) at 5° and 20°C. After 48 cycles of IML, no LHCII polypeptides were detectable at either 5° or 20°C. Trans-16:1 in PG had increased from 0 to 21 mol % at 20°C but only from 0 to 8 mol % at 5°C. Thus, the observed temperature sensitivity of rye thylakoid PG is exhibited independent of LHCII insertion into thylakoid membranes.

ONTOGENY OF BETULA ALLEGHANIENSIS - PISOLITHUS TINCTORIUS ECTOMYCORRHIZAE

Massicotte, H.B., C.A. Ackerley and R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario N1G 2W1

Seedlings of Betula alleghaniensis Britt. were grown in plastic growth pouches and inoculated with Pisolithus tinctorius (Pers.) Coker & Couch to form ectomycorrhizae. Structural modifications of the interface between the two symbionts were documented during ontogeny of the association. Cellular and subcellular changes occurred in both sumbionts and included proliferation of rough endoplasmic reticulum and mitochondria, elaboration of labyrinthine wall branchings in the fungus, and radial epidermal elongation and deposition of electron-dense material in the host. The number and overall structure of fungal nuclei were monitored in various phases of the ectomycorrhizal association.

FUNGAL MORPHOGENESIS IN ECTOMYCORRHIZAE FORMED BETWEEN *LACCARIA BICOLOR* AND TWO TREE SPECIES (*BETULA ALLEGHANIENSIS* AND *PINUS RESINOSA*)

Massicotte, H.B., L.H. Melville and R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Modified growth of fungal hyphae in contact with roots of a putative host may be an important factor in the establishment of a compatible symbiotic relationship. In this study, the ectomycorrhizae formed between *Laccaria bicolor* and two tree species showed similar patterns of altered fungal growth in the Hartig net. Hyphae growing between root cells became multi-lobed, increased in diameter and formed an elaborate, branching network. The extent of colonization and Hartig net architecture may be host dependent.

DEVELOPMENT OF *LACCARIA BICOLOR* FRUIT-BODY PRIMORDIA

Melville, L.H., H.B. Massicotte and R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Laccaria bicolor fruit-body primordia formed in growth pouches during ectomycorrhiza synthesis with *Pinus resinosa*. Primordium development was investigated using SEM from the first signs of hyphal aggregation to the formation of a recognizable macroscopic structure (1.0 mm in size).

Changes in hyphal growth occur after contact between two vegetative hyphae. This is followed by the appearance of multiple clamp connections and adjacent branches which curl and become swollen. The resulting body of hyphae, the stipe primordium, grows at right angles to the pouch surface and does not appear to be either geotropic or phototropic.

Eventually, a locus of growth develops at the terminal portion and becomes the pileus primordium. In the latest stage of primordium development a new locus of growth appears on the pileus initial which may be phototropic.

Zones of differentiated hyphae, extracellular mucilage production, and nuclear activity are distinguished using various stains in combination with light and fluorescence microscopy.

COMPARISON OF THE PROLAMIN FRACTIONS OF *ZEA MAYS* AND *ZEA MEXICANA* .

Hilary Nield and Ann Oaks Biology Department, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

Several theories on the origin of maize have stated that teosinte is the progenitor of maize. Prolamin fractions of *Zea mays* (maize) and *Zea mexicana* (teosinte) were extracted and analyzed to determine whether evolution resulted in modifications. Ouchterlony immuno-diffusion assays were run using the prolamin fractions as antigens and a polyclonal monospecific antibody prepared against a commercial zein. The precipitin lines fused indicating that the two prolamins were homologous. Nitrogen determinations revealed that teosinte contained two times more protein per g dry weight than maize seeds. They also indicated that about 50% of the total protein was zein in each case. The average weight of one teosinte seed was 49.1 mg of which 22.3% was protein, whereas the maize seed weighed 192.6 mg of which 12.2% was protein. The weight of the non protein portion of the seed was mainly carbohydrate. Thus the evolution of maize has resulted in seeds that contain larger relative amounts of carbohydrate.

INTERACTION BETWEEN THE ECTOMYCORRHIZAL FUNGUS PISOLITHUS TINCTORIUS AND ROOT HAIRS OF PICEA MARIANA

Thomson, J., L.H. Melville and R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario N1G 2W1

Root hairs are known to develop interactions between both nitrogen fixing bacteria and vesicular-arbuscular mycorrhizal fungi, but there have been few detailed reports of associations between root hairs and ectomycorrhizal fungi. This study documents the interaction between the root hairs of *Picea mariana* Mill. and the ectomycorrhizal fungus *Pisolithus tinctorius*.

Root hairs of *Picea mariana* are susceptible to "mantle-like" colonization by *Pisolithus tinctorius* hyphae in a zone about .5-4.0 mm from the root apex. Initial contact with the hair appears to be mediated by a fibrillar substance on the root hair surface. Hyphae grow in an acropetal direction around the root hair in a tightly interwoven pattern, eventually forming a "mantle". This is the first report of "mantle-like" structures on root hairs of ectomycorrhizae.

Observations indicate that root hairs may play an important role in the establishment of hyphae on the root surface during ectomycorrhiza formation.

DETECTION OF PROTO-ONCOGENES IN MAIZE. R. Zabulionis*, D.B. Walden*, B.G. Atkinson# and J.D. Procunier#, Department of Plant Sciences*, Department of Zoology#, The University of Western Ontario, London, Ontario, N6A 5B7.

The presence of genes that govern cell division and differentiation is being investigated in Zea mays. Heterologous animal oncogene probes (v-Ha-ras, v-Ki-ras, v-src, v-fos, c-myb, v-myc) were hybridized to restriction enzyme cut, Southern blotted plumule DNA under conditions which allowed for up to 28% mismatch. Human, yeast and E. coli DNA served as controls for the hybridization conditions used.

Two unique maize sequences homologous to the ras genes were detected. No cross homology was observed between the maize Ha-ras- and Ki-ras-related sequences under the hybridization conditions used. Northern blot analysis of plumule polyA$^+$ RNA showed two distinct Ha-ras transcripts. The v-src, v-myc and v-myb probes each detected a single unique sequence in maize while under similar conditions of stringency, no sequences homologous to v-fos were found. These probes did not detect any homologous sequences in E. coli DNA and all the reported homologous sequences in human and yeast DNA were observed. These results emphasize the evolutionary conservation of animal proto-oncogenes to the plant kingdom.
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NOTES